# The Investigation of Structure & Function for the Enzyme 4Q7Q

Tanashia Burrell, Mikaya Punzalan, and Dr. Julia Koeppe
Department of Chemistry, State University of New York at Oswego

## Abstract

The goal of our project is to determine the function of an enzyme for which the three-dimensional structure is already known. According to the Protein Data Bank (PDB), 4Q7Q is the crystal structure of a possible lipase from *Chitinophaga pinensis*. Computational sequence and structure alignments were used to predict the function and determine where the active site is in the 4Q7Q structure. We then expressed this enzyme in *E. coli* before isolating the cells by centrifugation and using sonication to break the cell membranes and release the enzyme of interest. We passed the collected supernatant through a Ni-NTA affinity chromatography column to isolate the His-tagged 4Q7Q. We used gel electrophoresis (SDS-PAGE) to analyze samples from the column in order to determine which of these contained our target enzyme. The Bradford assay technique was used to determine the concentration of the enzyme 4Q7Q. Chromogenic substrates were used for the enzymatic assays so that the function of esterase or lipase could be determined. We have demonstrated that 4Q7Q can hydrolyze *p*-nitrophenyl acetate, which supports its function as an esterase. We are using two different lipid substrates, *p*-nitrophenyl decanoate and *p*-nitrophenyl dodecanoate to test for lipase function. We are comparing the activity of 4Q7Q as a lipase using either bile salts or phospholipid vesicles to solubilize the lipid substrates.

## Introduction

According to the Protein Data Bank (PDB), the enzyme 4Q7Q contains two chains denoted with the letters A and B in its structure, and it is proposed to be a lipase. The structure was determined at the Midwest Center for Structural Genomics. We performed sequence and structure alignments using BLAST, Pfam, Dali, and Moltimate. Each alignment suggested that 4Q7Q is an esterase or lipase.

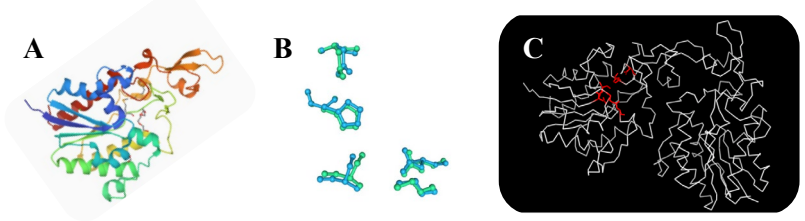


**Figure 1:** The structure and proposed active site of 4Q7Q. (A) 3D structure of the A chain of 4Q7Q. (B) Alignment of Gly69, Asn97, Asp29, Ser30, Asp251, and His254 from 4Q7Q (green) with the active site of 1BWP (blue), an acetylhydrolase. This alignment has an RMSD of 0.4725 Å. (C) Proposed active site of 4Q7Q shown in red on the A chain of the 4Q7Q homodimer (figure prepared using PyMOL). [1-3]

## Purification and Analysis of 4Q7Q

After completing the computational analysis, we prepared a sample of 4Q7Q in the lab in order to complete testing of the proposed lipase activity. A plasmid containing the gene for 4Q7Q was used to transform E. coli BL21(DE3) cells. We then grew transformed cells in autoinduction medium to produce the 4Q7Q enzyme. After collecting the cells and lysing them by sonication, we purified the 4Q7Q using NiNTA chromatography in which the His-tagged 4Q7Q bound to the column while other proteins flowed through. We used SDS-PAGE and a Bradford assay to analyze our purified sample.

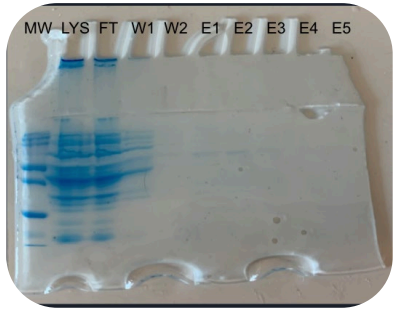


**Figure 2:** SDS-PAGE analysis of fractions from NiNTA chromatography. The samples that were loaded into the wells are 1) molecular weight marker 2) cleared lysate 3) column flow through 4) wash 1 5) wash 2, and 6-10) column elution fractions 1-5.

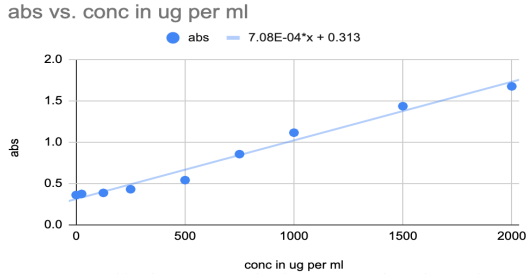


**Figure 3:** Bradford assay standard curve used to determine 4Q7Q concentration. From the linear fit of the standards, we determined that the concentration of 4Q7Q was 0.659 mg/mL.

## Acknowledgements

- SUNY Oswego Department of Chemistry
- Namrata Pokharel, Slater Bushen, and Christopher Serrano for assistance with production and purification of 4Q7Q.
- Dr.Kestutis G. Bendinkas

## Testing 4Q7Q Activity

Because 4Q7Q is predicted to be an esterase or lipase, we tested for enzyme activity using two chromogenic substrates. *p*-Nitrophenyl acetate (PNPA) is a small ester substrate, and *p*-nitrophenyl dodecanoate is a longer lipid substrate. Both substrates produce a yellow product, *p*-nitrophenolate ion, when hydrolyzed. Based on the linear fit of these graphs, the slope can be interpreted as the velocity of the reaction. This can be represented as $\frac{\Delta absorbance}{time}$ or $\frac{\Delta product}{time}$.

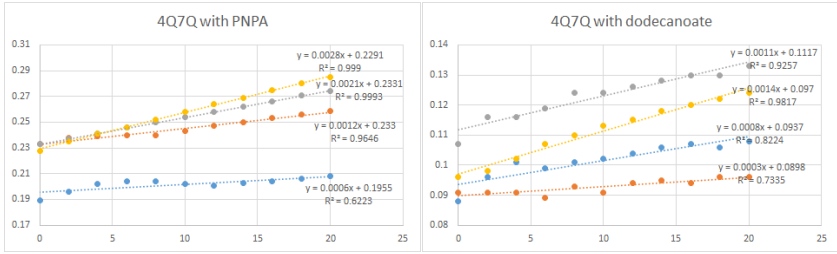


**Figure 4:** Kinetic analysis of 4Q7Q. Varying concentrations of 4Q7Q were tested to measure hydrolysis activity with two different substrates. The substrate concentration was the same for all trials, and 4Q7Q showed hydrolysis that was dependent on enzyme concentration with both substrates.

**Legend**
Blue: 0 mg/mL 4Q7Q
Orange: 0.05 mg/mL 4Q7Q
Yellow: 0.1 mg/mL 4Q7Q
Grey: 0.2 mg/mL 4Q7Q

## Future Work

Going forward with the investigation of structure and function of 4Q7Q we plan to test for activity with varying lipid substrate and constant enzyme concentration. This will be done by using 0.05 mg/mL 4Q7Q enzyme concentration with varying *p*-nitrophenyl dodecanoate from 0 mM up to saturating conditions (initial trials were 0-1.5 mM substrate concentration). Likewise, we will also be testing another lipid substrate, *p*-nitrophenyl decanoate. We hope to determine Michaelis-Menten kinetic parameters with other students who are testing ester substrates (*p*-nitrophenyl acetate and *p*-nitrophenyl butyrate).

## References

1. https://www.rcsb.org
2. moltimate.org
3. The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.